IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Jeffery P. Erickson

Serial No.:	10/505,191	Art Unit:	1632
Filed:	06/24/2005	Examiner:	Sgagias, M.
Entitled:	**Anticancer Compounds And Methods**		

DECLARATION OF DR. JEFFERY P. ERICKSON UNDER 37 CFR § 1.132

Mail Stop –Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Dated: September 26, 2007 By: Traci E. Light

Examiner Sgagias:

I, Jeffery P. Erickson, Ph.D. under penalty of perjury, state that:

1. I am the sole inventor of the embodiments of the invention as claimed in the United States patent application captioned above.

2. I understand that, in the Non-Final Office Action dated March 26, 2007 regarding the above captioned patent application, the Examiner has alleged that the specification does not provide adequate guidance for one to create a transgenic mammal secreting a heterologous protein into saliva.

3. I now provide data showing that, by using the teachings within the specification, a transgenic goat expressing the human serum albumin protein within its salivary gland was

successfully created. This project was performed under my direction by Dr. Ken Bondioli at Louisiana State University. In particular, a plasmid vector with a bovine salivary protein promoter (bSP30a) ligated to a human serum albumin protein was produced by conventional restriction techniques. Sambrook et al., In: Molecular Cloning, A Laboratory Manual, Vol. 1-3, Cold Spring Harbor Press (1989). Figure 1 shows the ligation strategy. Figure 2 shows insertion sites and PCR primer sequences. Figure 3 shows a flowchart demonstrating the overall plasmid construction process.

5. The constructed plasmid thereby places a bSP signal peptide sequence, a mature protein sequence, and a Poly A termination sequence all downstream of a bSP promoter sequence. The complete plasmid was transfected into a goat fibroblast and the transfected fibroblast was used for somatic cell nuclear transfer. See, Figure 4, top panel.

6. A transgenic doe was birthed that has been shown to be transgenic with transfected gene consisting of the bSP30a promoter and the human serum albumin gene. PCR analysis demonstrates that epithelial skin genomic DNA derived from the transgenic doe comprises both the bSP promoter sequence and the human serum albumin transgene. See Figure 4: Gel A – the bSP promoter sequence; Gel B – the human serum albumin gene; and Gel C – the linked bSP promoter – human serum albumin gene plasmid. DNA = genomic doe DNA; Plas = transfection plasmid; and Neg = negative control (i.e., no nucleic acids).

7. Secretion of the human serum albumin into the transgenic doe's saliva shows that the transgene is functional and expressed in the salivary gland.. A saliva sample was collected from the transgenic doe (Tg) and a non transgenic doe (non Tg) of similar age and analyzed by gel electrophoresis and Western Blot. Both saliva samples (15 µl each), an hSA standard (2 µg), a goat serum sample (3 µl) and molecular weight markers (M) were separated by electrophoreses on two identical 10% poly acrylamide gels. One gel was stained with Commassie Blue (CB) to show all proteins and the second gel used for Western Blot. Separated proteins in the second gel were transferred to a nitrocellulose membrane and the membrane incubated with a primary antibody consisting of a mouse monoclonal antibody against hSA. The membrane was then incubated with a secondary antibody consisting of a goat anti mouse IgG antibody coupled to the

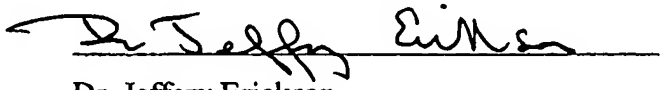
Horseradish Peroxidase enzyme. Western Blot bands were visualized with the BCIP/NBT color detection system.

8. In the first Western Blot analysis (see Figure 6 and the corresponding CB stained gel Figure 5) the membrane was incubated with the anti hSA antibody for 20h. This analysis clearly shows a single immuno stained band in the Tg saliva with is barely noticeable in the non Tg saliva sample and co migrates with a major band in the hSA standard. Even though the goat serum lane is overloaded (Figure 5) the anti hSA antibody does not stain any protein in goat serum (Figure 6).

9. In the second Western Blot analysis (see Figure 8 and the corresponding CB stained gel Figure 7) the incubation with the anti hSA antibody was reduced to 2h. This significantly reduces the background staining seen after a 20h incubation. An immuno staining band is clearly seen in the Tg saliva and no corresponding band is seen in the non Tg saliva. Again, even though the goat serum lane is overloaded (Figure 7) no immuno stained band is seen (Figure 8).

10. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: September 26, 2007



Dr. Jeffery Erickson

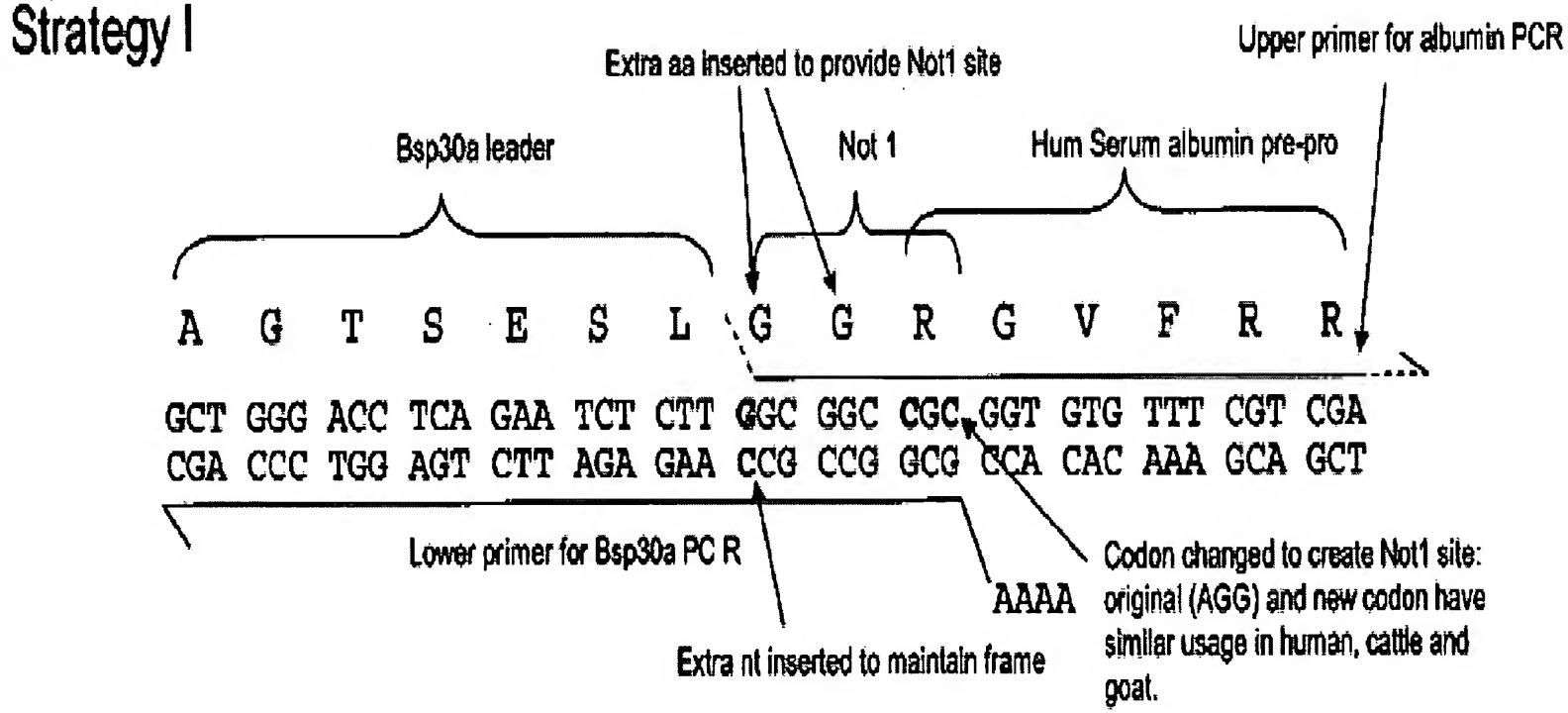


FIGURE 1

PCR Primers Incorporating Restriction Sites by 5' Mismatch

Not I Sac I Primers for Human Serum Albumin

```
cacgcctttg     gcacaatgaa     gtgggtaacc     tttatttccc     ttctttttct     ctttagctcg
gcttattccaggggtgtgtttcgtcgagat           gcacacaaga     gtgaggttgc     tcatcggttt
     gcggccgc   ggtgtgtttcgtcga

aaagatttgg     gagaagaaaa     tttcaaagcc     ttggtgttga     ttgcctttgc     tcagtatctt
cagcagtgtc     catttgaaga     tcatgtaaaa     ttagtgaatg     aagtaactga     atttgcaaaa    //


2161   gaagttccag     tgttct     ctcttattccacttcgg          tagag   gatttctagttt
                                    gagaataaggtgaagcc       at ctc   gag
```

Figure 2

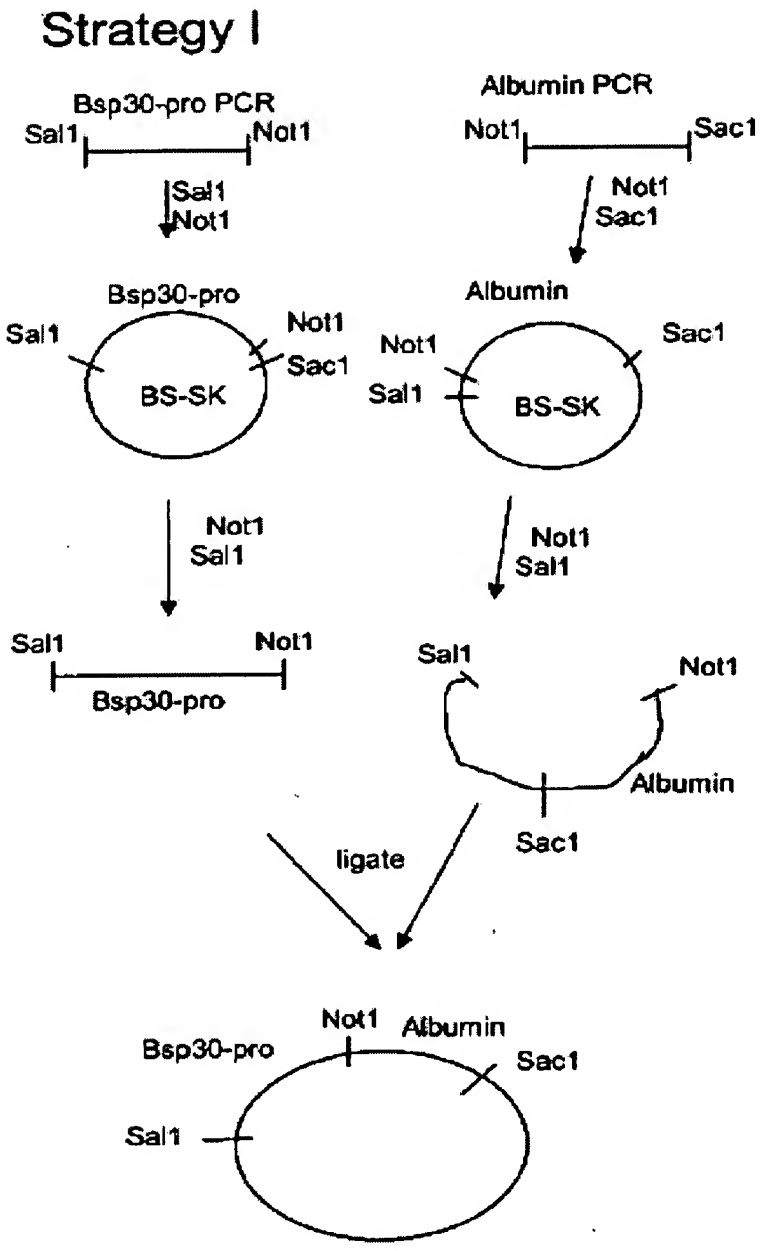

Strategy I
Bsp30-pro PCR
Sal1
Not1
Sal1
Not1
Albumin PCR
Not1
Sac1
Not1
Sac1
Bsp30-pro
Sal1
Not1
Sac1
BS-SK
Albumin
Not1
Sac1
Sal1
BS-SK
Not1
Sal1
Not1
Sal1
Sal1
Not1
Bsp30-pro
Sal1
Not1
Albumin
Sac1
ligate
Not1
Albumin
Bsp30-pro
Sac1
Sal1

Figure 3

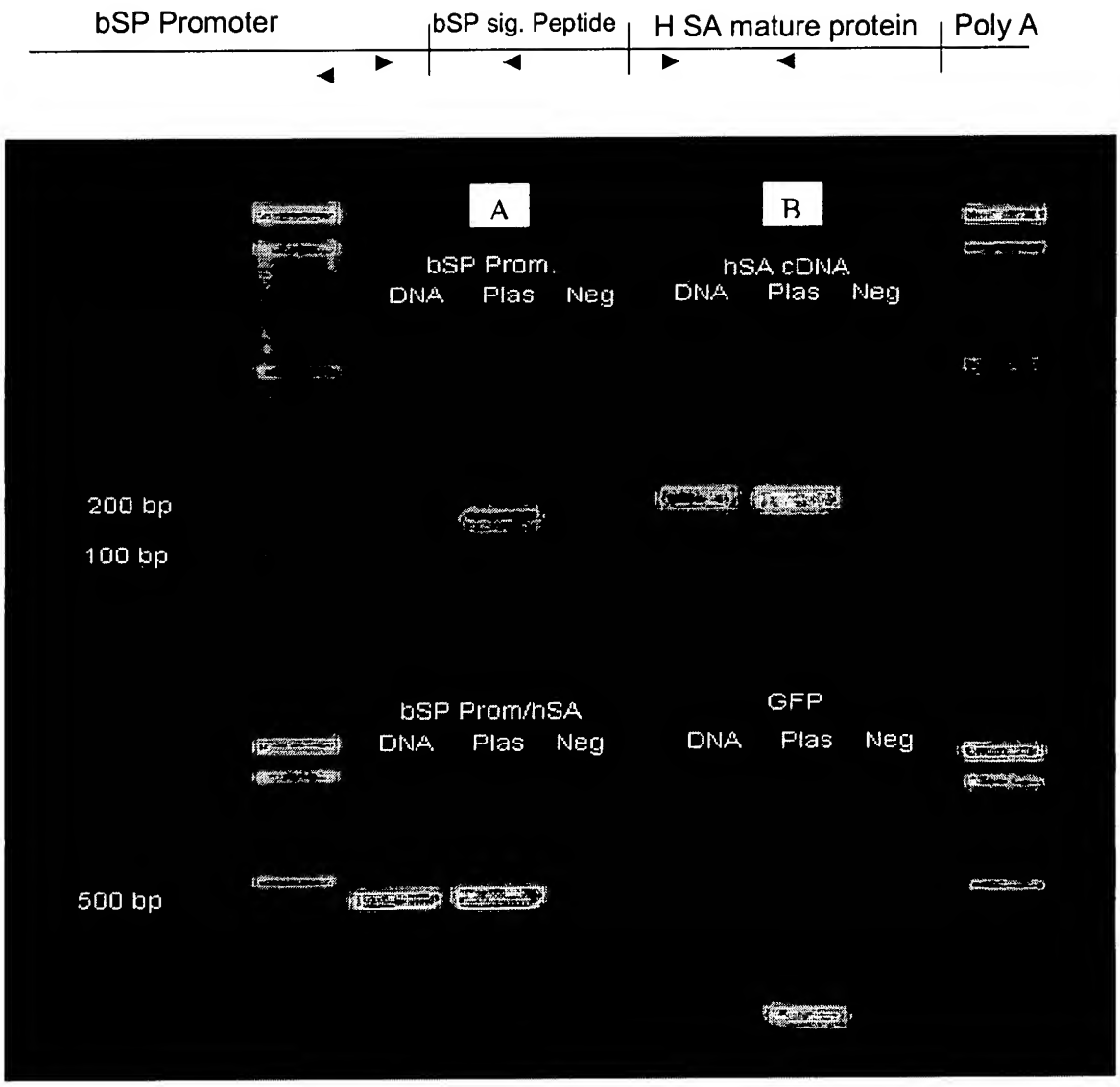

bSP Promoter
bSP sig. Peptide
H SA mature protein
Poly A
A
B
bSP Prom.
DNA Plas Neg
hSA cDNA
DNA Plas Neg
200 bp
100 bp
bSP Prom/hSA
DNA Plas Neg
GFP
DNA Plas Neg
500 bp

Figure 4

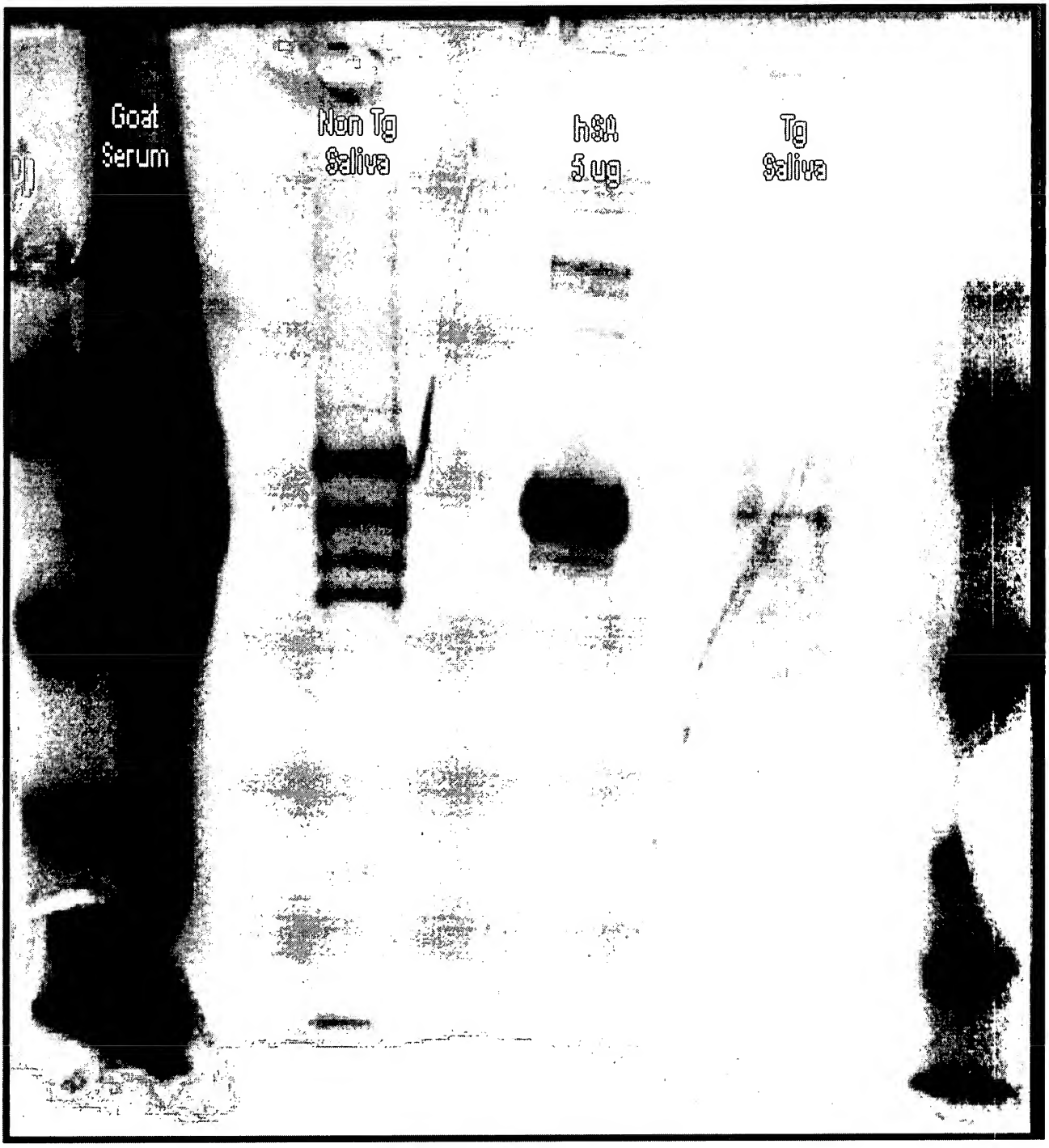


Figure 5
Commassie Blue Stain

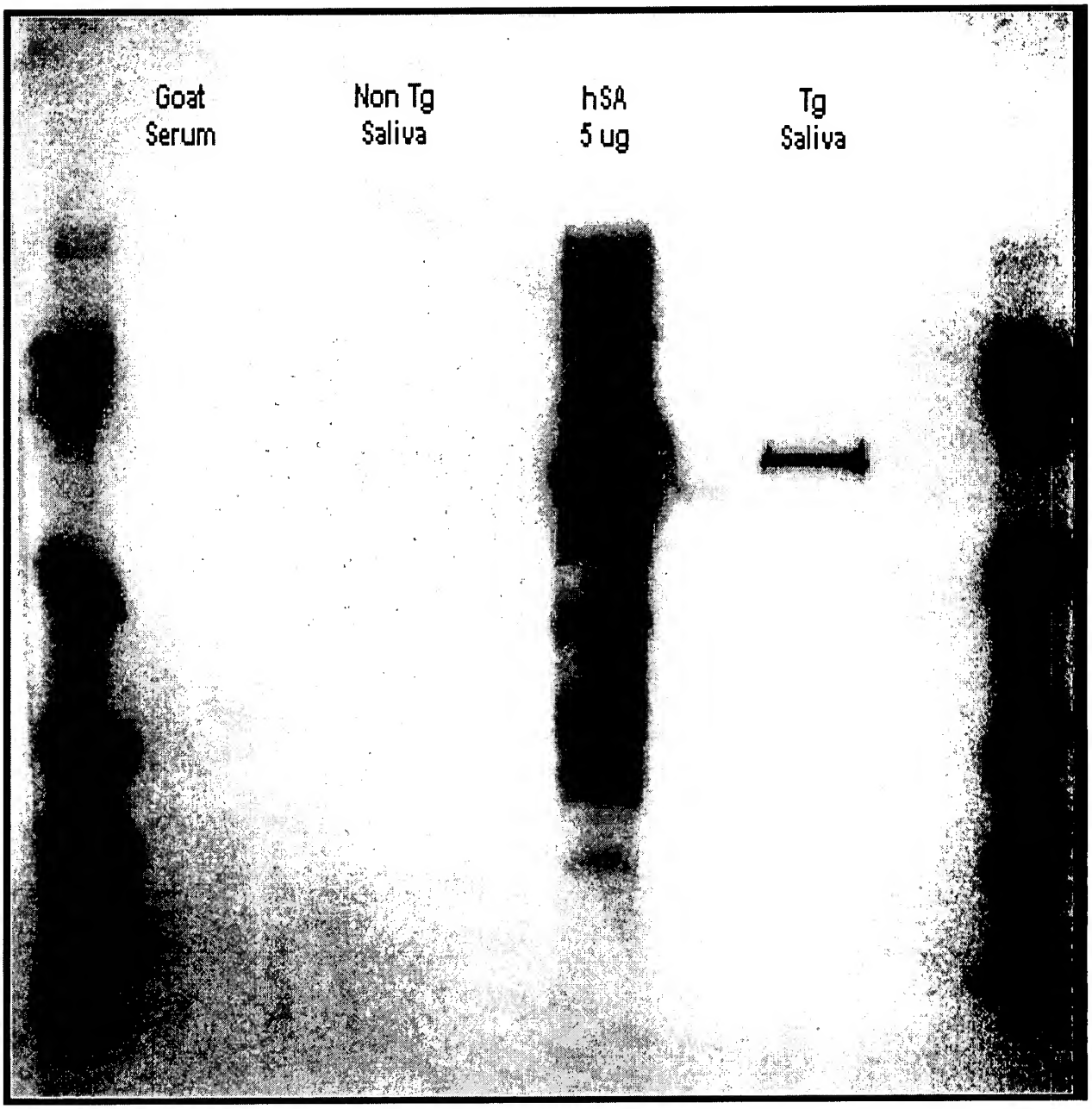


Figure 6
Horseradish Peroxidase-Mouse MAb anti-hSA For 20 hours

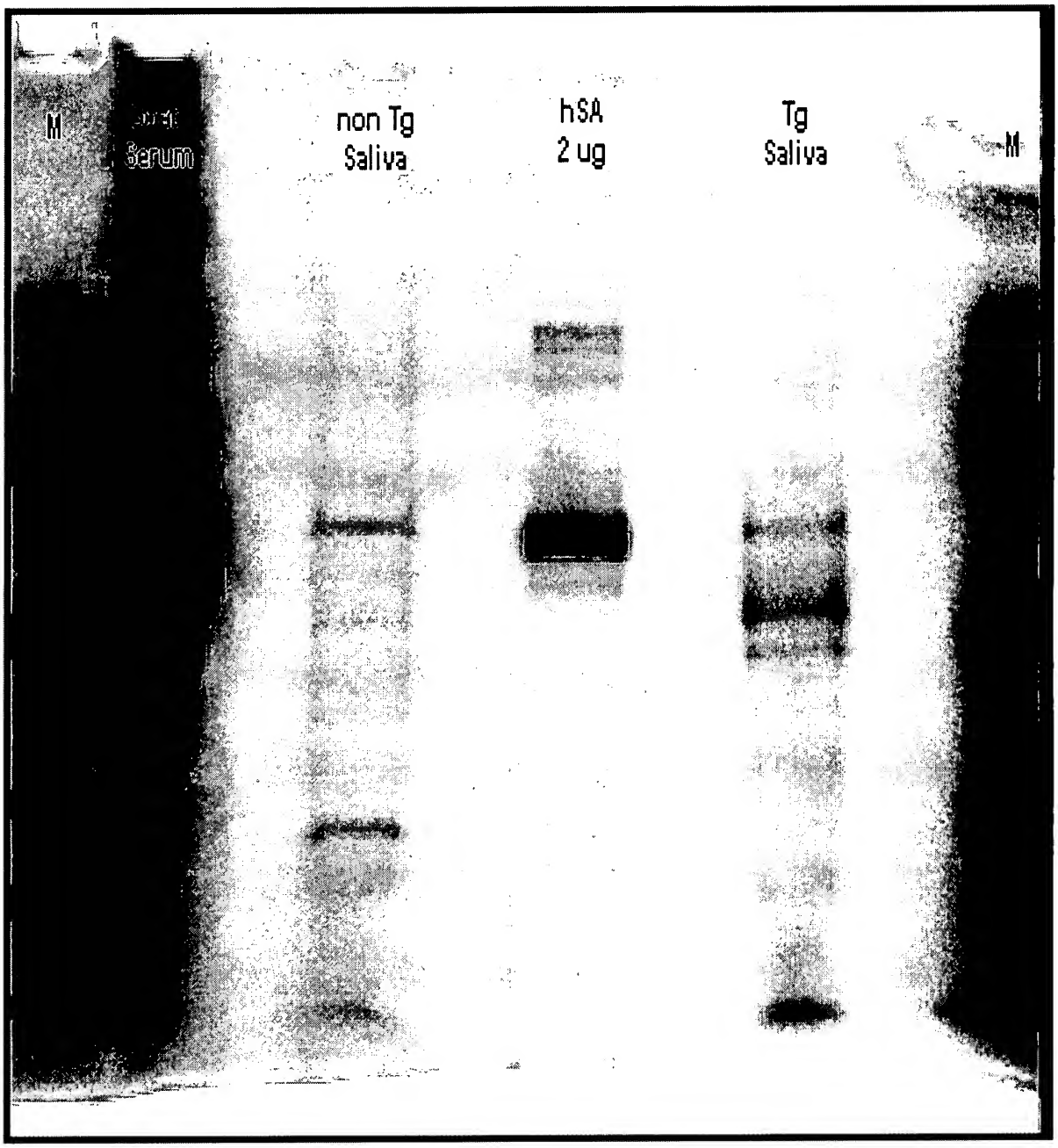


Figure 7
Commassie Blue Stain

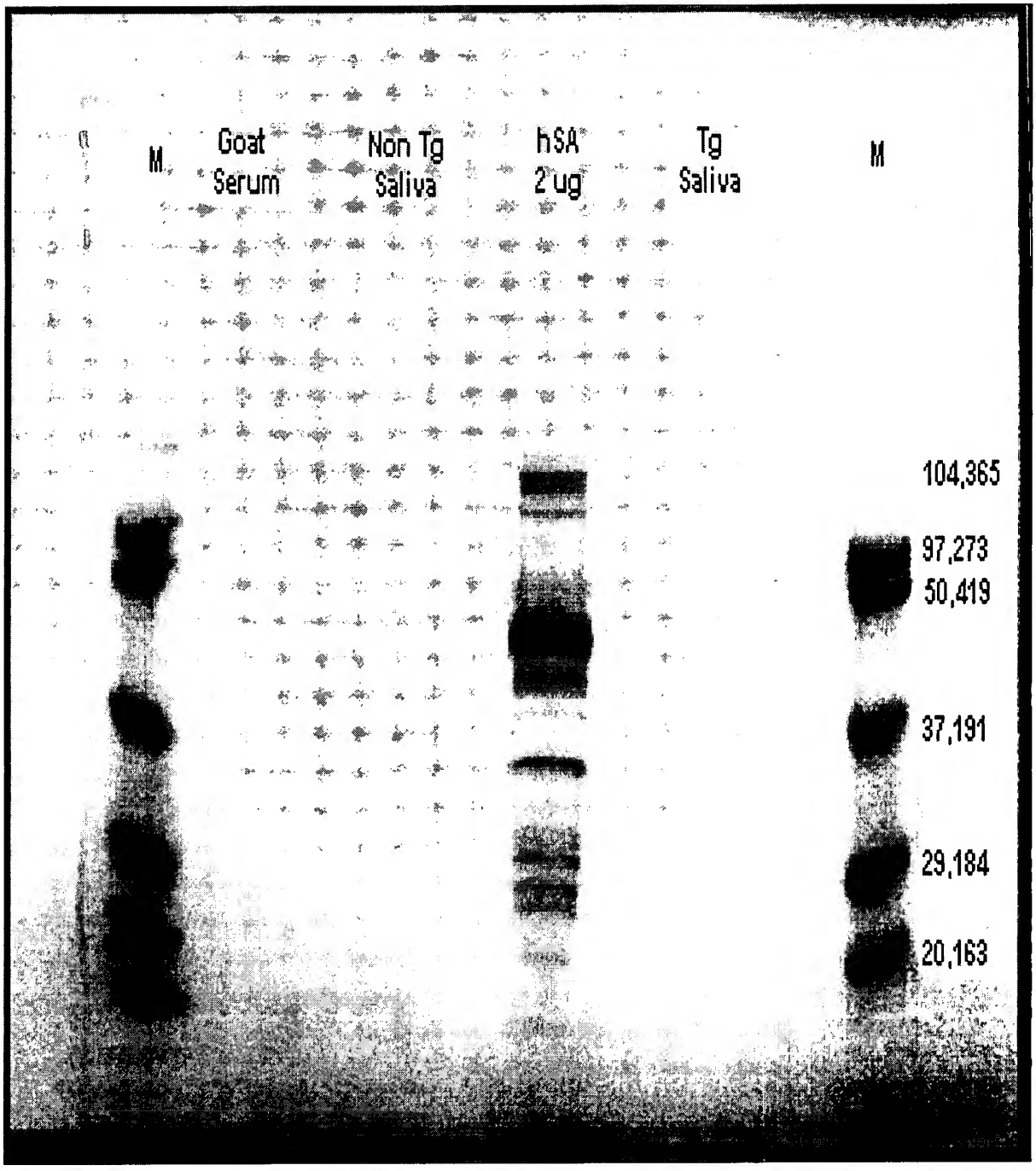


Figure 8
Horseradish Peroxidase-Mouse mAb Anti-hSA For 2 Hours